Nanoparticles and Ocean Optics

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LONG-TERM GOALS

Our long-term goal is to understand the role of the two most abundant nanoparticles in the sea, viruses and polymer aggregates and their impact on the inherent ocean optical properties of seawater.

OBJECTIVES

There are two objectives to this work that deal with the role of nanoparticles and ocean optics:

1. To determine the importance of viruses (and other biological processes) in the formation of chromophoric dissolved organic matter (CDOM) in the ocean through lysis of host cells.

2. To quantify the importance of aggregate formation to seawater optical properties.

APPROACH

The initial focus for the virology portion of this project (start date January ’05) was the acquisition and characterization of virus/host assemblages for use in lab-based dilution experiments. We tested methods for the separation of naturally occurring virus and host from seawater samples in preparation for dilution experiments with natural assemblages. Next, we examined the role of viruses in production of CDOM using a modified version of the Landry dilution method (Landry and Hassett 1982; Landry 1993; Landry, Kirshtein et al. 1995; Evans, Archer et al. 2003). Rather than using the dilution technique to focus on phytoplankton growth and mortality from grazing, we targeted CDOM production by all living cells, using UV absorption as the indicator of CDOM production. We tested this dilution approach on heterotrophic bacterial hosts and their specific viruses, in simplified experimental systems, to see if we could see CDOM release induced by viral lysis and if we could control infection by the dilution process. Subse-

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14. ABSTRACT
Our long-term goal is to understand the role of the two most abundant nanoparticles in the sea, viruses and polymer aggregates and their impact on the inherent ocean optical properties of seawater.

15. SUBJECT TERMS

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quent dilution experiments have included eukaryotic hosts (*Emiliania huxleyi*) and its specific viruses. We then did experiments with field samples from the Bigelow Laboratory dock as well as from shipboard experiments.

WORK COMPLETED

In the first year of this project, we focused on transitioning our laboratory experiments to the field, where we applied the Landry dilution technique and diluted field samples containing a variety of hosts with virus-free seawater. We separated natural sea water assemblages off the Bigelow Lab dock into a concentrated suspension of free viruses (20-200nm), and a fraction with particles >200nm that contained mostly hosts (prokaryotes and eukaryotes). This was accomplished using tangential flow ultrafiltration, which enhanced the concentration of only particles <200nm (such as viruses) in one fraction and concentrated larger "host" cells (like phytoplankton, heterotrophic flagellates, heterotrophic dinoflagellates, ciliates, etc.) in another. For the actual dilution experiment, we diluted the "host" particles with virus-free seawater, hypothesizing that the some fraction of the hosts were already be infected with viruses, which means we have not only the hosts, but their specific viruses in our concentrated suspension. Dilution of these infected hosts should then regulate the subsequent infection cycles of the viruses, achieving our goal, which is to regulate natural virus infection via dilution and better understand its optical consequences. Instead of making diluent from 0.2 μm-filtered seawater, we made it with 0.02 μm-filtered seawater, to insure the diluent was virus-free. Ultra-filtration has been used before to sequester and concentrate free-floating viruses in natural assemblages so that one could examine associated effects on their hosts (Suttle, Chan et al. 1990; Proctor and Fuhrman 1992; Suttle 1992; Peduzzi and Weinbauer 1993; Weinbauer and Peduzzi 1995; Noble, Middelboe et al. 1999). The difference here is that we applied Landry's dilution method using diluent prepared with ultrafilters that have 10X smaller porosity than used previously. Ultimately, this allowed derivation of rates of production of CDOM associated with particles in the virus size range (e.g. viruses). Time courses of absorption, scattering, fluorescence, particle size distribution and virus concentration were performed. The simplicity of this experimental design is that we were able to directly regulate the role of viral lysis in the production of CDOM.

This project also involved the examination of the optical properties of nanometer-sized aggregates in experiments in which we augmented 20 nm filtered seawater with natural, filter-sterilized CDOM. We then incubated this mixture in the presence or absence of sodium azide (to inhibit biological reactions) or EDTA (to regulate chelation, which controls aggregate formation) (Chin, Orellana et al. 1998) and parallel measurements of volume scattering and absorption were performed. Shipboard experiments were performed with this same basic experimental design, except no augmentation of DOC was made to the samples and the experiments were run with only the naturally-occuring DOC. Optical changes were followed using WETLabs ac-9s measuring spectral absorption and attenuation, as a function of time.

During the second year, we completed processing of our field data from the *R/V Cape Hatteras* cruise. Moreover, we also began experiments on a new virus that we isolated from the Gulf of Maine. This virus (ø 43) infected the coccolithophore, *Emiliania huxleyi*, at a rapid rate that we have never observed in a eukaryotic virus. Optical experiments demonstrated major optical changes on the time scale of hours (described below) instead of days (which has been the norm in previous work). We also devoted time towards publishing the results of our work on the in-

fection of various strains of the prokaryote, *Synecchococcus* sp., and associated optical consequences. Following the completion of that work, we are preparing the next paper on the optical consequences of viral infection of a eukaryotic phytoplankton species, *E. huxleyi* which will include these new exciting results.

During year three of this project, we have primarily focused on publication of results from the laboratory experiments as well as the field campaigns.

RESULTS

Virus characterization (Vaughn)
A collaborative paper involving our observations from the viral characterization experiments was submitted (Vaughn, Novotny et al. 2008). The paper describes how viruses infecting the marine coccolithophore, *Emiliania huxleyi*, were isolated from waters of the Gulf of Maine during May and June of 2004, a period when ambient *E.huxleyi* concentrations were minimal. Three DNA-containing, ether-resistant isolates possessed icosahedral symmetry, and were 130-160nm in diameter. The results demonstrated that all isolates caused complete lysis of host cultures within four days, produced large plaques on host lawns in agarose and were highly stable at -72^{o}C. Originally propagated on the 88E strain of *E.huxleyi*, none of the isolates was able to infect related strains of this organism, including several that were native to the Gulf of Maine. They were also noninfectious for species of *Synechococcus* and *Micromonas pusilla.* Lastly, we demonstrated that single-step growth studies showed a high level of virus propagation during the first 24 hr, a period when corresponding host populations were quite stable. This finding led us to speculate that during this period, progeny might be leaking from intact host, with eventual host lysis occurring later. Virus infections were extremely efficient, with a multiplicity of infection (MOI) as low as 10^{-5}affecting total lysis of host cultures within four days. Similar efficiencies were seen when host/virus concentrations were as low as $10^{2}ml^{-1}$. An additional isolate, which appeared to possess similar characteristics to the above viruses, exhibited an unusually rapid growth cycle, with host lysis occurring within 10 hr post infection.

Optics (Balch)
A second paper on the impact of viruses on the optical properties of four Synechococcus strains was published in the third year of this project, in Limnology and Oceanography (Balch, Vaughn et al. 2007). A third paper on the impact of viruses on the optical properties of *Emiliania huxleyi* is in preparation (Balch, Vaughn et al. 2008). Two more papers on controlled optical observations of natural aggregation of micron-sized PIC particles, plus the primary fates of such particles in the mixed layer are currently in revision for JGR (Balch, Plueddeman et al. 2008; Pilskaln, Dam et al. 2008). Another paper on the physics associated with particle dispersion through the mixed layer is in preparation. (Plueddemann, Balch et al. 2008). Lastly, an invited paper, on the impact of ocean acidification on particle optics, specifically related to PIC particles, has been revised for Marine Ecology Progress Series (Balch and Fabry 2008).

Aggregation observations (Goes)
As with the above efforts, work this year has been primarily focused on preparation of results for publication. Specifically, we have been focusing on experiments performed aboard the *R/V Cape Hatteras* cruise during year two of the project. Few data exist to examine the optical consequences of aggregation on processes such as optical backscattering. Polymer gel aggregation

has previously been thought to be common in the marine environment but over the course of our work we have observed a) that optical backscattering responds on time scales of days to changes in aggregation beginning with ultra-filtered seawater, b) that indeed chelation inhibits aggregation and c) the associated backscattering increases even in the presence of sodium azide. Other experiments were performed at sea in which instruments for measuring optical absorption and attenuation (WETLabs ac-9's) were used as incubation vessels, for the continuous incubation and optical monitoring of 0.2μm filtered seawater (either untreated or in the presence of sodium azide). These experiments were performed at three sites in the Gulf of Maine. Measurements of the size distribution of nanometer-size particles (using a flow, flow field fractionator) demonstrated that aggregation did occur from 50nm to 200nm size range in the azide-killed control (Fig. 2). The results of experiments of the three shipboard experiments, revealed a role for both biotic and abiotic factors in the formation of colloidal particle aggregates in seawater. In Experiment 3, for example (Fig. 2C), the large increase in particle aggregation within the sodium azide-treated samples as opposed to the control samples, suggested that abiotic factors were more important. In experiment 2, however, aggregation was more apparent in the untreated, control volume (Fig. 2B)

IMPACT/APPLICATIONS

This work is important to our understanding of the impact of viral infection on the optical properties of seawater and its suspended microbial communities. In particular, it is critical to know the magnitude of changes in inherent optical properties, along with the rates of change and host specificity associated with viral infection. Our field and laboratory dilution experiments have provided this. Along with changing the size spectrum of the particulate matter, viruses essentially convert Case I waters (where the optical properties can be easily indexed to chlorophyll *a*) to Case II-like waters (where the optics are dominated by dissolved organic matter, not chlorophyll *a*). Results of this work have provided important understanding of the time scales of optical changes for the numerically-abundant nano- and picoplankton following infection. The discovery of the virus ø 43 demonstrates that viral infection rates of eukaryotic hosts are even faster than we had originally postulated. Our long-term goals are to better understand time-space optical variability associated with viruses, which ultimately provides improved observational capabilities, ocean color algorithms, and predictive-multidisciplinary models. This work also is important for understanding the optical properties associated with polymer-gel formation. Polymer gels begin as nanometer-sized colloids of dissolved organic matter (DOM) that aggregate, producing micron-sized colloids. Polymer gels and viruses represent two of the most abundant particles in the sea; thus, our results have far-reaching implications for ocean optical properties, for both molecular and particle scattering.

TRANSITIONS

Our dilution experiments have shown the power of the technique for understanding the role of virus infection in ocean optics. However, complex microbial communities, when diluted, are still complex microbial communities. In future experiments, we plan to use flow cytometry sorts to simplify viral-host interactions in natural populations and better elucidate their impact on ocean optical properties.

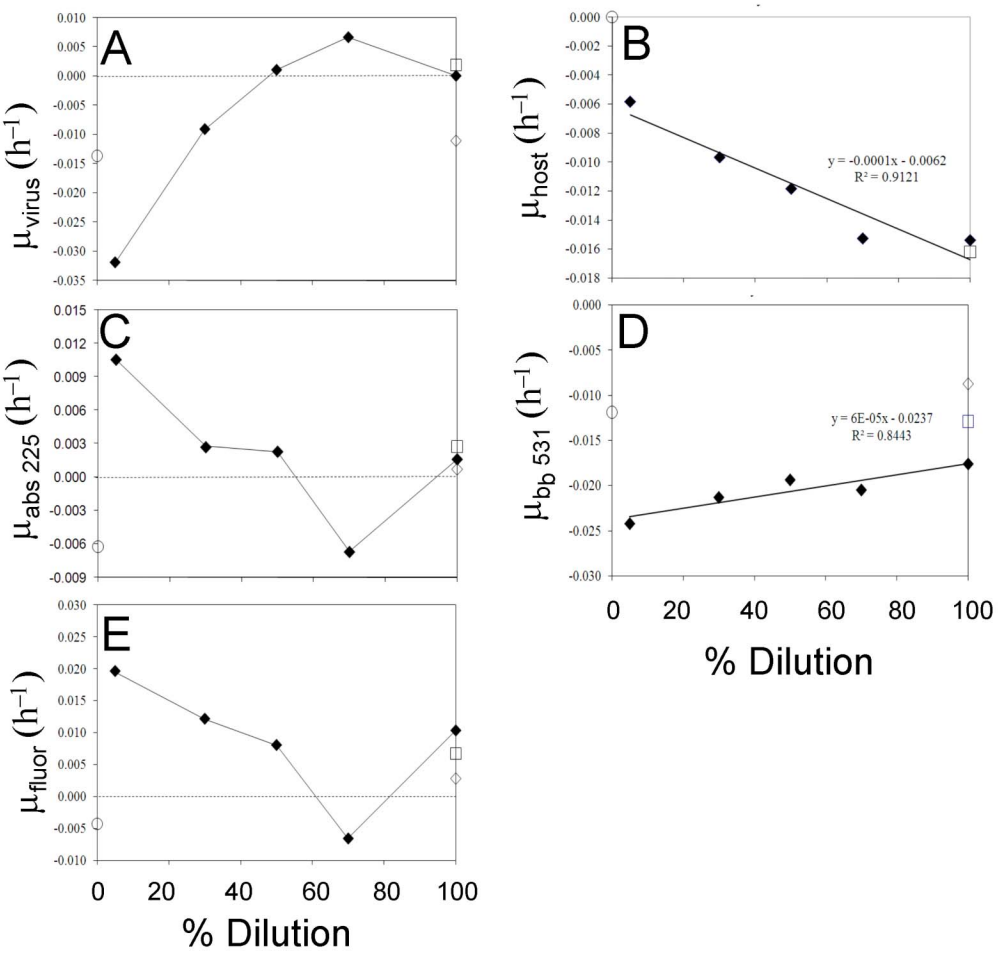

Fig. 1-Results of virus dilution experiment in which a natural population from surface waters of the Gulf of Maine was run through a 990Kdalton tangential flow cartridge in order to concentrate all host cells. A subsample of this concentrate was then filtered through a 0.2μm polycarbonate (in order to remove host cells, leaving only free viruses). Free viruses then were concentrated by putting the 0.2μm-filtrate through a 50 Kdalton tangential flow cartridge. The major assumption of this experiment was that the 990 Kdalton suspension was assumed to contain host cells, some of which were infected with their conspecific viruses. Dilution of these host suspensions with virus-free (0.02μm-filtered) seawater would then reduce the subsequent infection rate of the host cells accordingly (Landry 1993; Evans, Archer et al. 2003). Indeed, our assumption was correct since host cells diluted with virus-free seawater, showed the appearance of viruses in several hours. A) Plot of dilution versus virus abundance showed that virus concentrations increased at highest rates in the 70% host suspensions. The exact same pattern was reproduced in multiple ship-board experiments. Peak intrinsic rates of viral increase were 0.006 per hour (0.14 per day). At 50 and 100% dilutions, viral propagation rate was quasi stationary while at 30 and 10% dilutions, viral propagation decreased with time. One explanation for the lower intrinsic propagation rate of viruses at the most concentrated dilution may have been due to the impact of multiple infection while at lower

dilutions, the viruses would have had less likelyhood finding their hosts. B) Plot of host intrinsic growth rate versus dilution. It can be seen that the intrinsic growth rate of the hosts was linearly related to the dilution rate, with the steepest decrease in hosts associated with highest virus/host concentrations. Moreover, the Y intercept at zero dilution represents the natural host mortality rate in the absence of viruses. C) Rate of change of UV absorption (225nm) plotted against dilution. The trend is inverse to the virus growth rate. That is, the greatest loss rates of UV absorption were seen at highest virus propagation rates and greatest increases in UV absorption at low virus growth rates. D) Rate of change in backscattering at 531nm plotted against dilution. Note the increased rate of increase of backscattering in most concentrated host suspensions, inverse to the host growth rates. It appears that viral-induced mortality of host particles is associated with increased backscattering. E) Rate of change in chlorophyll fluorescence plotted against dilution. Again, the rate of change in fluorescence mirrors the UV absorption, with the lowest rate of increase in chlorophyll fluorescence (indeed a loss) seen at the 70% dilution. Symbols for all panels: black diamonds= diluted host cells; open circles = ultra-filtered seawater controls; open squares = host + added free virus; open diamond = 100% free virus only.

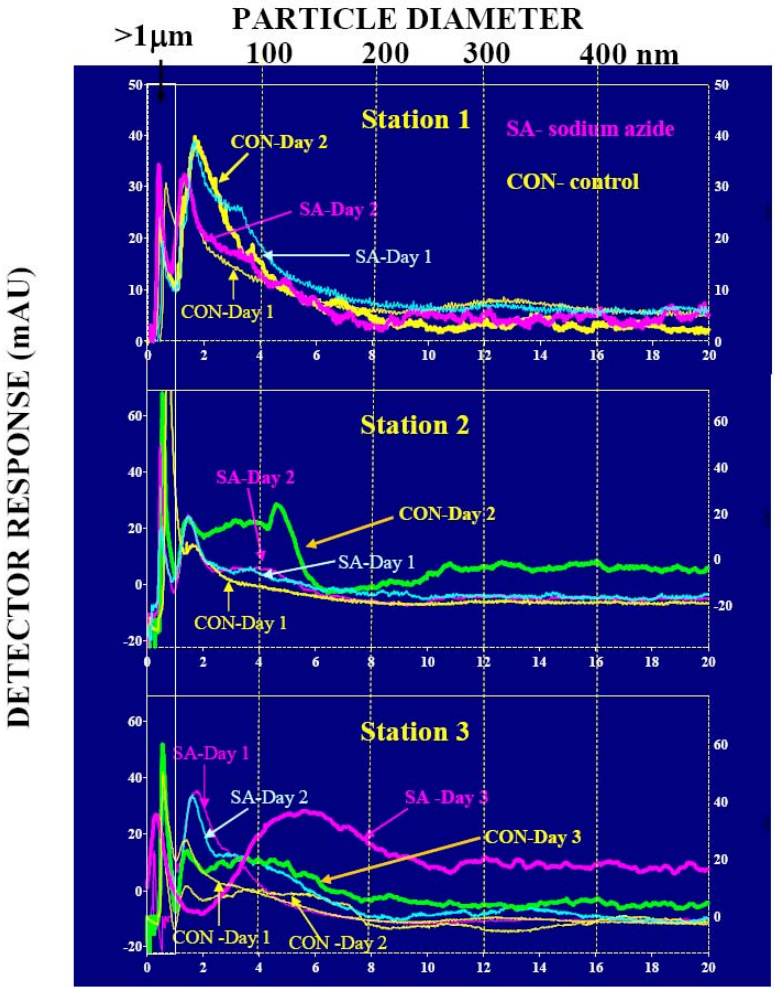

Figure 2- FFFF fractograms showing particle size changes in sodium azide ("SA") treated and untreated (control or "CON") samples of seawater obtained from three stations (St. 1- river discharge dominated, St. 2 -coastal and St. 3 -offshore oligotrophic) within the Gulf of Maine. Both the treated and control samples were first pre-filtered through a 0.2μm pore size filter and then separately circulated through an ac-9 to quantify changes in optical properties resulting from colloidal particle aggregation and/or consumption.

Particle aggregation was observed in all three experiments, in both the control and in the treated samples regardless of the water types associated. With the exception of the experiment undertaken at Station 1, there were subtle differences in aggregation between the controls and the sodium-azide treated samples. In all cases, the 0.2μm pre-filtered seawater samples at the commencement of each experiment (Day 1), were generally dominated by colloidal particles in the 40-80 nm size range, but by the end of the experiment changed as follows: A) At station 1, the pre-filtered samples were characterized by two peaks, the primary peak associated with 50-80 nm particles and the secondary minor peak of 300-400nm size particles. In the sodium-azide treated sample, approximately 24 hrs through the experiment, there was an

increase in particles of the size range between 200 and 300nm. This increase was associated with a decrease in 50-80 nm and 300-400nm size particles. In the control samples, FFFF fractograms at the end of the 24 h circulation period presented a contrasting picture. Here an increase was observed in the 50-80 nm particle size class which possibly could have been caused by bacterial metabolism of particles within the 300-400nm. B) At station 2, the control sample witnessed the most conspicuous change in its particle size spectra at the end of the experiment. At the start of the experiment, the colloidal pool of the control sample comprised largely of ~40nm size particles, but was dominated by particles between 40nm and 150 nm (peak at ~120 nm) by the end of 24 hour experiment. A notable but less conspicuous increase was observed in particles of the >200nm size range. The sodium-azide treated samples in contrast, witnessed a small increase in ~110 nm particles, but no change was apparent in the intensity of the 40nm size particles peak. C) The experiment at Station 3 lasted over a period of 48 h. Over this period, particle aggregation was observed both in the treated and in the control samples. At the end of the 48 h period, the largest changes in particle size distribution were observed within the treated samples as a result of the conspicuous increase in colloidal particles of the 100 to 200 nm size range. In the control samples, an increase was observed of particles ranging in size between 50 and 150 nm at the end of the 48 h, but note that the magnitude of this increase was smaller than the treated samples. The extension of this experiment to 48 h over and above the 24 h length of Experiments 1 and 2 allowed us to observe a further increase in particle sizes of colloids and their magnitude over time.

The results of experiments 1-3, reveal a role for both biotic and abiotic factors in the formation of colloidal particle aggregates in seawater. In Experiment 3, in particular, the large increase in particle aggregation within the sodium azide treated samples as opposed to the control samples, suggests that abiotic factors were more important for particle aggregation.

RELATED PROJECTS

This ONR grant supplied funds for a high sensitivity digital microscope camera/stage manipulator, which has been used for high-resolution virus viewing with SYBR-stained viruses (a fluorescent DNA stain). We have developed software for a) automated focusing for microscopic samples and image acquisition of phytoplankton samples and b) image analysis algorithms (in conjunction with the Univ. Massachusetts Computer Sciences Department through a separate NSF grant) for automated analysis of particles based on their fluorescence or birefringence properties. The automated phytoplankton enumeration software is now running 24h per day, as we are processing thousands of samples from our field work and experiments. A complete description of this system will be published in the peer-reviewed literature.

The state of Maine has awarded us an autonomous Slocum glider which we are using for understanding the distribution of colored dissolved organic matter, particularly in the Gulf of Maine. This is a subset of the total dissolved organic matter, the source material of polymer gel aggregates studied in this project. As of this writing, we have just completed our first 22d, cross-Gulf mission, providing a new look at the three-dimensional CDOM distribution at extraordinarily high resolution, along with backscattering, chlorophyll fluorescence and spectral downwelling irradiance and upwelling radiance at 5 wavelengths across the visible.

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